EFFECT OF UREA ON BACILLUS COLI, BACILLUS TYPHOSUS AND STAPHYLOCOCCUS AUREUS.

by

Matthew E. Carroll

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Approved by:

Noble P. Sherwood.

Department of Bacteriology.

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INTRODUCTION

In 1919 Sherwood[1] observed the effect of urea in streptococcus infections of rabbits. He determined the minimum fatal dose for rabbits of an hemolytic streptococcus when administered intravenously and observed that when 1 to 3 percent of urea was added to the suspension, the animals survived. Blood agar plate controls showed that the streptococci were living at the time of injection. In attempting to explain the observed phenomenon, it occurred to him, that the mechanism of urea hemolysis described by Eisenberg[2], and later by Kosakai[3], might be true for bacteria. In attempting to verify the work of Kosakai, he discovered that the quantitative relationship given by him was not general, but held for sheep and beef erythrocytes, and that human and rabbit cells required a much more

concentrated solution of urea for the hemolytic dose. Sherwood further observed that similar results occurred quite commonly, but not uniformly when working with hemolytic streptococcus in vitro.

The work of this paper may then be sub-divided as follows:

1. A critical review of the experimental work done by Sherwood.

2. An extension of this phenomenona to determine whether it holds for Bacillus Coli, Bacillus Typhosus, and Staphylococcus Aureus.

3. To determine the concentration of urea that is germicidal for these organisms under certain definite conditions.

4. To determine the effect of varying hydrogen-ion concentration on reduction.

Sherwood, in his experiments, observed that while there was a variation in the concentration of the urea necessary to bring about hemolysis of erythrocytes of different species, there was practically no variation among members of the same species. Blood from any number of rabbits was used. The rabbits were of all sizes, ages and conditions.

No effort was made to control the animals. The blood from only one sheep was used in the course of the experiments. The human cells were obtained from many of the students, both men and women. The primary purpose of the experiments was to obtain as many samples of blood as possible from as many different suorces as possible.

TECHNIC

Technic used was that of Kosakai.[3] He states that if 0.05 cc. of corpuscular suspension treated for ten minutes with 0.8 cc. of 10 per cent urea, the total volume being 1 cc., the sudden addition of 1 cc. of physiological salt solution would produce very strong hemolysis, while the addition of 2 cc. of the solution would cause complete hemolysis.

The corpuscle suspension in each case in these experiments was prepared by defibrinating whole blood, centrifuging, pipetting off the serum and washing the cells three times with physiological salt solution. The cells were then made up to the original volume with .85 per cent NaCl solution.

The concentrations of urea were made up volumetrically in marked 100 cc. bottles. The urea used was Merek's C.P. product. Sterile physiological (.85%) NaCl solution was used as a solvent.

The test tubes used in determining cytolysis tests were of ordinary american glass, and in size, 9 x 1 cm., inside measurements. Small hemolytic test tubes were found to be unsatisfactory.

All the experiments were carried out at room temperature, 20° to 25°C. Slight temperature changes were negligible.

(1) To each of three test tubes, add 0.05 cc. of corpuscle suspension (sheep cells). To a fourth tube, add the same amount. This tube is used for a control. The use of three tubes gives more reliable results.

(2) The contents of test tubes 1, 2 and 3 were treated with 0.8 cc. of a 10 per cent solution of urea. The total volume in all four tubes made up to 1 cc. with .85 per cent NaCl colution.

(3) To all four tubes, add suddenly, 1 cc. of .85 per cent NaCl solution. Results recorded as in Set A_1, Table 1. A_1, Table 1, shows the effect of

adding 1 cc. of NaCl after cells have been in contact with urea for ten minutes.

(4) Set up another set of tubes in like manner, but instead of adding 1 cc. of .85 per cent NaCl solution as a diluting fluid, add 2 cc. suddenly, by blowing from a pipette. As a result, complete hemolysis occurs in the first three tubes, whereas when 1 cc. of salt solution was used as a diluting fluid, strong, but incomplete hemolysis resulted.

The above experiments were repeated using 9, 8, 7 and 5 per cent of urea solution, respectively. The same technic was used on human and rabbit red blood cells.

The results of the experiments on sheep cells are tabulated in tables 1, 2, 3 and 4.

Table I

10 per cent concentration of urea on sheep cells

	Tube	A_1 Hemolysis	A_2 Testing time
Set A	1	S	10 min. S
	2	S	S
	3	S	S
	4(control)	-	-
Set B	1	Alc	C
	2	C	C
	3	C	C
	4(control)	-	-

c--Hemolysis complete.

Alc--almost complete.

s--strong hemolysis.

Set A, 1 cc. isotonic salt solution added.

Set B, 2 cc. isotonic salt solution added.

Table II

9 per cent concentration of urea
on sheep cells.

	Tube	Hemolysis	Treating time 10 min
Set A	1	S	S
	2	S	S
	3	S	S
	4(control)	*	-
Set B	1	C	C
	2	C	C
	3	C	C
	4(control)	-	-

c--hemolysis complete

Alc--almost complete

s--strong hemolysis.

Set A, 1 cc. isotonic salt solution added.

Set B, 2 cc. isotonic salt solution added.

Table III

8 per cent concentration of urea
on sheep cells.

	Tube	Hemolysis	Treating time 10 min.
Set A	1	S	S
	2	S	S
	3	S	S
	4(control)	-	-
Set B	1	C	C
	2	C	C
	3	C	C
	4(control)	-	-

C--hemolysis complete

Alc--almost complete

S--strong hemolysis

Set A, 1cc. isotonic salt solution added.

Set B, 2cc. isotonic salt solution added.

Tables 2 and 3 show that the lowering of the concentration 1 or 2 per cent causes no perceptible difference in the degree of hemolysis.

Table IV

5 per cent concentration of urea used.

Tube	Hemolysis	Treating time 10 min
1	Trc	Trc
2	Trc	Trc
3	Trc	Trc
4(control)	-	-
1	S	S
2	S	S
3	S	S
4(control)	-	-

Trc--trace of hemolysis

s--strong hemolysis

Set A, 1cc. isotonic salt solution added.

Set B, 2cc. isotonic salt solution added.

Table IV shows that lower concentration of urea (5 per cent) decreases the amount of hemolysis preduced. A 20 per cent concentration of urea hemolyzed human and rabbit cells under the same conditions of experimentation. When homologous serum was used instead of isotonic salt solution as a diluting fluid as in experiments shown in Tables I, II, III and IV,

hemolysis was obtained where indicated in Tables I II, and III, and noohemolysis in Table IV. A 50 per cent solution of the hemologous serum and isotonic salt solution gave no different results than had been obtained with salt solution alone.

II. Effect of the lowering of the concentration of solution on Bacillus Coli, Bacillus Typhosus, and Staphylococcus Aureus.

TECHNIC

1. The growth of two 24-hour agar slants of Bacillus Coli was emulsified with 4 cc. of 4 per cent urea solution, two cc. of the 4 per cent urea added to each tube.

2. Suspension from both tubes transferred to sterile tube, and allowed to stand for 15 minutes.

3. One cc. suspension measured into tubes 1,2 and 3.

4. 2.5 cc. physiological saline added slowly to tube 2. 2.5 cc., 4 per cent urea added to tube 3.

5. Dilute in sterile water blanks, plate on nutrient plain agar.

6. Count plates after 24 hours' incubation at 37° C.

Table V

Plate counts when a 4 per cent concentration of urea was used.

6

Tube	1	2	3	4	Average	Bact. per cc.
1	49	39	42	53	47	470,000,000
2	70	75	676	77	72	720,000,000
3	98	109	78	99	91	910,000,000

Dilutions used 1-10 million.

The counts are from 4 series of plates.

1. NaCl solution (.85 per cent) added rapidly.
2. NaCl solution (.85 per cent) added slowly.
3. Urea (4 per cent) added.

Ratio of reduction, 1:2.

Table VI

Plate counts when a 4 per cent concentration of urea was used.

Tube	1	2	3	4	Average	Bact. per cc.
1	82	84	42	72	70	700,000,000
2	64	99	85	65	78	780,000,000
3	98	81	105	95	94	940,000,000

Dilutions used 1-10 million.

The counts are from four series of plates.

1. NaCl solution (.85 per cent added rapidly.
2. NaCl solution (.85 per cent) added slowly.
3. Urea (4 per cent) added

Ratio of reduction 1:1.5

Table VII

Plate counts when a 4 per cent concentration of urea was used.

Tube	1	2	3	4	Average	Bact. per cc.
1	49	43	38	40	42	420,000,000
2	23	30	12	24	22	220,000,000
3	26	31	33	18	24	240,000,000

Dilutions used 1-10 million.

Counts are from four series of plates.

1. NaCl solution (.85 per cent) added rapidly
2. NaCl solution (.85 per cent) added slowly.
3. Urea (4 per cent) added.

No reduction shown.

Table VIII

Plate counts when 4 per cent concentration of urea was used.

Tube	1	2	3	4	Average	Bact. per cc.
1	39	42	25	29	39	340,000,000
2	22	32	40	38	33	330,000,000
3	39	37	63	67	51	510,000,000

Dilution used, 1-10 million.

Counts are from four series of plates.

1. NaCl solution (.85 per cent) added rapidly.
2. NaCl solution (.85 per cent) added slowly.
3. Urea (4 per cent) added.

Rates of reduction 1: 16

Table IX

Plate counts when 4 per cent concentration of urea was used.

Tube	1	2	3	4	Average	Bact. per cc.
1	49	29	41	68	46	230,000,000
2	-	-	51	36	43	215,000,000
3	57	40	85	70	63	315,000,000

Dilution used, 1-5 million

Counts are from four series of plates.

1. NaCl solution (.85 per cent) added rapidly.
2. NaCl solution (.85 per cent) added slowly.
3. Urea (4 per cent) added.

Ratio of reduction, 1:1.4

Table X

Plate counts when a 6 per cent concentration of urea was used.

6

Tube	1	2	3	4	Average	Bact. per cc.
1	63	82	77	59	69	690,000,000
2	39	50	36	-	43	430,000,000
3	53	59	63	-	58	580,000,000

Dilution used 1-10 million

Counts are from 4 series of plates

1. NaCl solution (.85 per cent) added rapidly
2. NaCl solution (.85 per cent) added slowly.
3. Urea (6 per cent) added

Ratio of reduction .9:1

Table XI

Plate counts when a 6 per cent concentration of urea was used.

Tube	1	2	3	4	Average	Bact. per cc.
1	110	107	94	88	102	510,000,000
2	62	58	88	66	68	340,000,000
3	38	37	48	54	44	220,000,000

Dilution used, 1-5 million.

Counts are from four series of plates.

1. NaCl solution (.85 per cent) added rapidly.
2. NaCl solution (.85 per cent) added slowly.
3. Urea (6 per cent) added.

Ratio, 2:3:1

Tables X and IX show germicidal effect of urea on Bacillus Coli.

The reduction in these two tables is not in Tube 1 but in Tube 3. The same ratios of reduction found for Bacillus Typhosus and Staphylococcus Aureus.

A 3 per cent concentration gave the best reduction without germicidal effect for Bacillus Typhosus, while a 4 per cent concentration showed the highest ratio of reduction for Staphylococcus Aureus.

The best ratio of reduction obtained for Bacillus Typhosus was 1:4 and for Staphylococcus Aureus, 1:3.

Table XII

666

Organism	Per cent of urea causing greatest reduction	Ratio of reduction
B. Coli	4%	1:2
B.Typhosus	3%	1:4
Stl Aureus	4%	1:3

Table XII shows the best reduction obtained in the course of the entire experiment and the concentration of urea causing reduction.

A 6 per cent concentration of urea is germicidal for Bacillus Typhosus, as well as Bacillus Coli. At times a 5 per cent concentration appeared germicidal for Bacillus Typhosus. A 6 per cent concentration of urea is definitely germicidal for Staphylococcus Aureus.

III. Effect of varying Hydrogen-Ion Concentrations or Reduction of Bacillus Coli, Bacillus Typhosus and

Staphylococcus Aureus.

The concentration of urea used was that, found to be bacteriolytic, but not germicidal for the three organisms.

Using a 4 percent concentration of urea on Bacillus Coli:-

1. 4% urea solution P-H 9.0, salt solution, P-H 7.4= reduction.
2. 4% urea solution P-H 7.4, salt solution, P-H 7.4= reduction.
3. 4% urea solution P-H 7.4 salt solution, P-H 9.0_- reduction.
4. 4% urea solution P-H 9.0 salt solution, P-H 9.0_= reduction.

A 3 per cent concentration of urea on Bacillus Typhosus, a 4 per cent concentration of urea on Staphylococcus Aureus gave reduction regardless of variation in hydrogen-ion concentration.

DISCUSSION

Results in Table I show that a quantitative difference in the amount of NaCl solution added causes a marked difference in the degree of hemolysis produced.

In some cases, where the treating time was ten minutes, as in Set A_2 and B_2, Table I, an effort was made to control the time factor. The degree of hemolysis produced at first is not changed as shown by com-

paring the results before and after the lapse of 10 minutes. Because of these results, the treating time was not varied in the other experiments.

Sherwood[1] in his experimental work with urea and human cells found that a 10 per cent concentration of urea hemolyzed human red blood cells when the concentration of the surrounding medium was suddenly lowered, using different technic, than followed in these experiments.

Eisenberg[2], while not taking into account the varying concentrations of urea that produce hemolysis, observed briefly that in the lower concentrations of urea in physiological saline solution, the effect is produces by a sudden immersion of the treated corpuscles in isotonis salt solution. This investigator claims that a 5 per cent concentration of urea would in a given time produce a higher degree of hemolysis than could be obtained using a higher concentration of urea for a shorter time.

Wells[4] places the bacterial cell intermediate between the plant and animal cell, and states that bacteria are modified very easily by environment. In discussing bacteria and their osmotic relationship, he says that they can be plasmolyzed. Pfeiffer[5], in his work on Microspira Comma, speaks of the dissolving of lysis

sis

sis of the bacterial cells caused by specific immune s serum. In this paper the cytolysis spoken of, is similar in its mechanism to that of water hemolysis.

The results in tables V,VI,VIII and IX show the results of a 4 per cent concentration of urea treated Bacillus Coli when the concentration is suddenly lowweed, producing bacteriolysis, judging from the reduction in count.

The results can be better understood by the comparison of the number of bacteria per cc. in tubes 1 and 3 of Tables V,VI, VIII and IX. Tubes 1 and 3, before treatment, contained approximately the same number of bacteria. The ratios of reduction in Tables V, VI, VIII and IX are fairly constant, and point to direct reduction. In table V, the ratio of reduction is 1: 2. Therefore, for every bacterium in tube 1, there are two in tube 3. A glance at table V will show that in tube 1, the concentration of the solution (4 percent) was suddenly lowered with isotonic salt solution. Tube 2, should show the same results as tube 3. It does not, however. Regardless of how slow the diluting fluid is added, bacteriolysis does take place to some extent.

The results in tables IX and XI, show that a 6 per cent concentration of urea is definitely germicidal, under the conditions of this experiment. In tables X and XI, the reduction is in tube 3 and not in 1. This tube

having received quantitatively 2.5 cc. more of 6 per cent urea solution than tube 1. Table XI shows that a 6 per cent of urea is markedly germicidal for Bacillus Coli. Quite similar results were obtained using a 6 per cent concentration of urea for both Bacillus Typhosus and Staphylococcus Aureus.

Symmers and Aberd[6] find that a concentration of urea below 25 per cent has no decided bactericidal effect. In this report they did not say what organisms they were using to determine the bactericidal effect of urea.

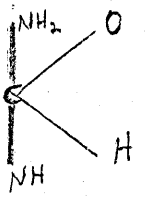
Wilson[7], found that when 8 per cent of urea was present in the media, there wass practically no growth of Bacillus Coli and that still lower percentages caused a number of the organisms when treated with low concentrations of various antiseptics, react in the same way.

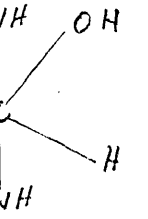
In the course of this experiment, I was unable to see that urea in concentrations up to 10 per cent caused any difference in the appearance of the Bacillus Coli colonies, Bacillus Typhosus or Staphylococcus Aureus on plain agar plates or agar slants. There is no abnormality in the growth or colony formation of urea treated Bacillus Coli on Eosin methylene blue plates in either the lower or higher concentrations of urea.

According to Cole[8], urea exisrs in two tautomeric

modifications, the equilibrium between them depending on the reaction.

Werner[9] says that there are two forms, A and B.

A 

B 

The A forms exist in strongly acid solutions and are decomposed by nitrous acid. The B forms exist in neutral or alkaline solutions. This form is not decomposed by nitrous acid.

In this experiment, the B form was used throughout, dissolved in isotonic salt solution, that has a P-H 7.4; the P-H of the solution runs up into the alkaline range, P-H of the solution runs up into alkaline range, P-H 9.0 for concentrations of 4 or 5 percent.

CONCLUSIONS.

I. Following the technic of Kosakai[3], the hemolysis of red blood cells of sheep, human and rabbit can be produced by the sudden lowering of the concentration of the corpuscular suspension. When the concentration of the suspension is slowly lowered, some hemolysis takes place, but complete laking does not occur. The concentration of urea necessary to cause hemolysis of red cells differs for different species.

2. The phenomena that Sherwood[1] found true for blood cells and hemolytic streptococcus, holds good for Bacillus Coli, Bacillus Typhosus and Staphyloccus Aureus.

3. The reduction or bacteriolytic action of urea on Bacillus Coli, Bacillus Typhosus and Staphyloccus Aureus can be determined only when the concentrations of urea used are not decidedly germicidal. For Bacillus Coli, the concentration of urea giving the best ratio of reduction is 4 per cent, for Bacillus Typhosus 3 per cent, and for Staphylococcus Aureus a 4 per cent concentration.

4. Under certain definite conditions, of experimentation as carried out in this paper, a 6 per cent concentration of urea is germicidal for Bacillus Coli, B Bacillus Typhosus and Staphylococcus Aureus.

5. Varying hydrogen-ion concentration for the urea solution and the isotonic salt solution used in lowering the concentration of the bacterial suspension has no effect and does not alter the ratio of reduction.

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